UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

May 25, 2012

MEMORANDUM

SUBJECT: **Review of an Alga Growth Inhibition Study for the Section 3 Registration of Zestat A-100 containing Ceytlpyridnium Chloride as the active ingredient.**

PC Code: 069160	DP Barcode: 400810
Decision No.: 431761	Registration Nos.: 83402-1
Petition No(s).: NA	Regulatory Action: Ecological Environmental Review of Studies for Section 3 Registration
Risk Assessment Type: Single chemical	Case No.: NA
TXR No.: NA	CAS No.: NA
MRID Nos.: 48012106	40 CFR: None

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RASSB has reviewed one study for the Section 3 Registration of Zestat A-100. The study was a 72-hr alga growth inhibition test with *Selenastrum capricornutum* under static conditions. It was found to be Supplemental and the results from the study can be used in a risk assessment.

DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

1. **CHEMICAL:** Cetylpyridinium chloride **PC Code No.:** 069160

2. **TEST MATERIAL:** Cetylpyridinium chloride **Purity:** 94.8%

3. **CITATION**

Author:	Knight, B.; Murphy C.M.
Title:	Alga, Growth Inhibition Test (72 h, EC_{50})
Study Completion Date:	March 2, 2005
Laboratory:	Inveresk Tranent, EH33 2NE, Scotland
Sponsor:	Rutherford Chemicals, PO Box 730, Route 611, Delaware Water Gap, PA 18327
Laboratory Report ID:	23189
Study Report ID:	804209
MRID No.:	48012106

4. **REVIEWED BY:**

Signature: **Date:** 5/25/12

David Bays, Microbiologist, RSSAB, AD

5. **APPROVED BY:**

Signature: **Date:** 5/25/12

Donna Randall, RSSAB, AD

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Selenastrum capricornutum*
Definitive Test Duration:	72 hours
Study Method:	Static
Type of Concentrations:	Nominal and measured at test initiation only

7. **CONCLUSIONS**

A 72-hour static test using *Selenastrum capricornutum* was conducted to evaluate the acute toxicity of Cetylpyridinium Chloride (CPC) to freshwater algae. The study was adequately performed and included 3 replicates and a negative control and for each of seven exposure levels. Measured concentrations of two stock solutions (10 and 32 mg/L CPC) at test initiation were 9.65 mg ai/L and 29.2 mg ai/L, respectively, with recoveries of 90 to 89 percent of nominal.

Results Synopsis:

Growth Rate:	Hour	EC_{50} (mg ai/L)	95% Confidence Interval (mg ai/L)	NOEC (mg ai/L)
	24	1.2	NP*	0.1
	48	36.8	NP	0.1
	72	26.9	NP	3.2
Area Under Curve: (AUC)	24	NC	NP	--
	48	3.2	NP	--
	72	9.3	NP	3.2

*Not Presented as Pearson's chi-square statistic was statistically significant
-- lowest concentration tested was significantly different from control

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: The test was run following the OECD guideline 201 recommendation of 72 hours instead of the EPA guideline requirement of 96 hours.

C. Repairability: This shorter running time for the algal test did not significantly affect the final results. The test was long enough to demonstrate the toxicity of CPC to freshwater algae. Therefore, these results can be used in a risk assessment.

9. GUIDELINE DEVIATIONS

- Cell density of 6.04 $x10^5$ cells/mL at 72 hours was below the Guideline recommendation of 3.5 x 10^6 cells/mL at 96 hours.
- Guideline used to prepare freshwater algal medium was not included.
- pH was 0.4 unit above the high end of the Guideline recommended range (at test initiation) and 0.7 units above the high end (at test termination) of the range.
- Quality of dilution water was not specified.
- No information was provided for photsynthetically active radiation.
- The test was run for 72 hours instead of the EPA guideline recommendation of 96 hours.

10. SUBMISSION PURPOSE: Section 3 Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ *Selenastrum capricornatum (Raphidocelis subcapitata)* ▪ *Skeletonema costatum* ▪ *Anabaena flos-aquae* ▪ *Navicula pelliculosa*	▪ *Selenastrum capricornutum*
Initial Number of Cells ▪ 10,000 cells/mL (*Selenastrum*)	▪ 10,000 cells/mL
Stock Culture ▪ 3 to 7 days old	▪ 3 days old
Nutrients ▪ Standard formula (ASTM E1218-20) ▪ pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletonema*) ▪ Freshly prepared	▪ ISO freshwater algal medium (p 27) ▪ pH 7.9

B. Test System

Guideline Criteria	Reported Information
Solvent ▪ Upper limit - 0.5 mL/L	▪ No solvent/carrier
Temperature ▪ 24° ± 2°C (*Selenastrum*) ▪ Recorded hourly	▪ Ranged from 23 to 24°C ▪ Measured daily.
Light Intensity ▪ 4.3 K lx (± 10%) (*Selenastrum, Skeletonema, Navicula*) ▪ Photosynthetically active radiation approx. 66.5 ± 10% µEin/m^2/sec	▪ Ranged from 7.7 K lx ▪ Photosynthetically active radiation not provided
Photoperiod ▪ Continuous (*Selenastrum*)	▪ Continuous
pH ▪ 7.5 ± 0.1 (*Selenastrum*) ▪ Measured at beginning and end of test	▪ Ranged from 7.9 to 8.2 ▪ Measured at beginning and end of test
Oscillation Rates ▪ 100 cycles/min (*Selenastrum*)	▪ 100 cycles/min
Test Containers ▪ 125-500 mL Erlenmeyer flasks ▪ Cleaned/sterilized (solvent and acid) and conditioned ▪ Test solution volume ≤ 50% of flask volume	▪ 250 mL Erlenmeyer flasks ▪ Test solution was 100 mL

Dilution Water ▪ Sufficient quality (e.g., ASTM Type I) ▪ Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	▪ Quality not specified

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test ▪ Water solubility and physical-chemical properties of test chemical determined? ▪ Validated analytical method developed? ▪ Expose algae to widely spaced (e.g. log interval) chemical concentration series ▪ Lowest value should be at detection limit ▪ Upper value, for water soluble compounds, should be at saturation concentration ▪ Minimum of 3 replicates ▪ Algae should be exposed for 96 hours ▪ If highest concentration (saturation concentration or 100 mg/L) results in <50% reduction in growth, definitive test may not be necessary ▪ If lowest concentration (detection limit) results in >50% reduction, definitive test necessary	▪ Data concerning water solubility, vapor pressure, and other physical/chemical properties were not available. ▪ Validated method ▪ Nominal test concentrations were selected in consultation with the Sponsor and were based upon results of an exploratory range-finding toxicity test. ▪ Definitive test justified based on results from range-finding test
Dose Range ▪ 1.5X -2X progression	▪ 2X progression
Doses ▪ 5 or more concentrations of test substance in a geometric series ▪ > 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	▪ 7 concentrations of test substance in a geometric series ▪ > 90% cell-density inhibition at highest concentration ▪ 100% growth (biomass) inhibition at highest concentration
Controls ▪ Negative and/or solvent each test ▪ Positive - zinc chloride (periodically)	▪ Negative control ▪ No positive control
Replicates Per Dose ▪ 3 or more (4 or more for *Navicula*)	▪ 3 replicates per dose
Duration of Test ▪ 96-hr	▪ 72 hours
Growth ▪ Logarithmic growth (controls) by 96-hr or repeat test ▪ 3.5×10^6 cells/mL (*Selenastrum*)	▪ Logarithmic growth was demonstrated in controls by 72 hours. ▪ 6.04×10^5 cells/ml at 72-hr

Daily Observations?	▪ Yes, test medium samples were collected from each replicate and control group at 24-hour intervals
Method of Observations ▪ Direct - microscopic cell count of at least 400 cells/flask ▪ Indirect - spectrophotometry, electronic cell counter, dry weight, etc; calibrated by microscopic count ▪ Qualitative and descriptive	▪ Direct: Compound light microscope an Improved Neubauer Counting Chambers
Cell Separation ▪ Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula)*	▪ No information provided on cell separation
Algistatic and algicidal effects differentiated?	▪ Algistatic and algicidal effects not differentiated

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Quality assurance and GLP compliance statements included.
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	Scientific name, source, method of verification and strain provided.
Growth in controls reported?	Yes
Description of test system and test design included?	Yes
Initial and final chemical concentrations and pH measured?	Yes
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	Yes
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	Yes
Raw data included?	Yes
Methods and data records reported?	Yes
Statistical Analysis ▪ Mean and standard deviation calculated and plotted? ▪ Goodness-of-fit determined?	▪ Yes, mean and standard deviation calculated and plotted ▪ Yes, Goodness-of-fit determined

Dose Response

Table 1. Summary of Cell Density after 24, 48, and 72 hours						
Nominal Concentration (mg ai/L)	Time 0 Measured concentration (mg ai/L)	Cell Density ($x10^4$ cells/mL)				Percent Inhibition
		24-hr	48-hr	72-hr		
Negative Control	<LOQ [a]	4.4	16.6	60.4		--
0.1	NA	2.6	12.6	71.7		0
0.32	NA	2.0	6.8	71.5		0
1.0	NA	2.5	15.1	59.1		2
3.2	NA	2.4	16.0	43.8		27
10	9.65	2.4	13.9	40.1		34
32	29.2	1.2	5.5	11.0		82
100	NA	0.3	0.2	0.3		100

[a] Less than limit of quantification of 0.0100 mg ai/L.

Table 2. Summary of Area Under Curve after 24, 48, and 72 hours						
Nominal Concentration (mg ai/L)	Time 0 Measured concentration (mg ai/L)	Biomass ($x10^4$ cells/mL)				96 hr Percent Inhibition
		0-24 hr	0-48 hr	0-72 hr		
Negative Control	<LOQ [a]	1.7	5.6	16.2		--
0.1	NA	0.8	3.7	16.2		0
0.32	NA	0.5	2.0	14.0		14
1.0	NA	0.7	4.3	14.9		8
3.2	NA	0.7	4.5	12.6		22
10	9.65	0.7	3.9	11.3		30
32	29.2	0.3	1.4	3.3		80
100	NA	0.0	0.0	0.0		100

[a] Less than limit of quantification of 0.0100 mg ai/L

Table 3. Summary of Growth rate after 24, 48, 72, and 96 hours						
Nominal Concentration (mg ai/L)	Time 0 Measured concentration (mg ai/L)	Growth Rate ($days^{-1}$)				96-hr Percent Inhibition
		0-24 hr	0-48 hr	0-72 hr	0-96 hr	
Negative Control	<LOQ [a]	14.6	13.9	13.6		--
0.1	NA	9.4	12.6	14.2		0
0.32	NA	6.5	9.6	14.2		0
1.0	NA	8.8	13.5	13.6		0
3.2	NA	8.4	13.8	12.6		7
10	9.65	7.9	13.2	12.2		10
32	29.2	3.1	8.3	7.8		40
100	NA	0.0	0.0	0.0		100

[a] Less than limit of quantification of 0.0100 mg ai/L

Statistical Results

Statistical Methods: The AUC and growth rate values were analyzed separately for homogeneity of variance using Levene's test at a 1% significance level. If no homogeneity of variance, the AUC and growth rate values were then analyzed using analyzed of variance (ANOVA) techniques. Following ANOVA, pairwise comparisons were performed between the control and concentrations using a one-tailed Dunnett's test at the 5% significance level. If there was heterogeneity of variance, the AUC and growth rate values were to be transformed using a log transformation. The goodness of fit of the probit model to the AUC and growth rate data was checked via the Pearson chi-squared test statistic.

13. VERIFICATION OF STATISTICAL RESULTS

The EC_{50} and NOEC calculations were inspected for reasonableness with respect to the raw data. Calculations of cell density averages and standard deviations were checked for accuracy.

14. REVIEWER'S COMMENTS:

Only one significant guideline deviation based on EPA OPPTS Guideline 850.5400 was found for this study. The running time for the study was only 72 hours instead of the proscribed 96.